

REMARKS

Upon entry of the present amendments, claims 1, 17, 27-33 and 46 will be pending. Claims 34-45 have been canceled without prejudice.

Claims 17 and 27 have been amended to recite methods for treating ischemic diseases of the heart, kidney, liver, or brain. Support for these amendments is found in the specification as filed at least at page 20, lines 30-31.

New claim 46 has been added. Support for new claim 46 is found in the specification as filed at least at page 187, line 29 to page 188, line 8 where an *in vitro* JNK inhibition assay is described.

No new matter has been added. Applicant reserves the right to prosecute the subject matter of any canceled, amended or withdrawn claim, or any other unclaimed subject matter, in one or more continuation, divisional or continuation-in-part applications.

I. The Double Patenting Rejection

Claims 1, 17 and 27-45 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being allegedly unpatentable over claims 13, 14, 24-27, 38 and 39 of copending Application No. 10/004,642 (the “’642 application”). Applicant submits herewith a Terminal Disclaimer Under 37 C.F.R. § 1.321(c) with provision for the required fee (in duplicate).

Accordingly, Applicant respectfully submits that the double patenting rejection over the ’642 application has been overcome and should be withdrawn.

II. The Rejections Under 35 U.S.C. §112, First Paragraph

Claims 1, 17 and 27-45 are rejected under 35 U.S.C. § 112, first paragraph, for being allegedly indefinite. In particular, while acknowledging that the specification is enabling for the “inhibition of the JNK pathway,” the Examiner has stated that treating the conditions recited therein are not enabled.

Preliminarily, Applicant notes that claims 17 and 27 have been amended to recite methods for the treatment of ischemic diseases of the heart, kidney, liver, or brain. In other words, these claims are directed to a specific class of disease which, as evidenced by the previously submitted literature references (*i.e.*, Bennett, *et al.*, *P.N.A.S.* 98(24):13681-13686 (2001), previously submitted as reference DH), is associated with JNK. Applicant further notes that claim 1 only recites treating conditions responsive to inhibition of the JNK pathway.

Using the Examiner's reasoning set forth in the present Office Action, a method of treating a disease with a compound would not be patentable until after the completion of a clinical trial. Such is not the law. *In re Brana*, 51 F.3d 1560, 1567 (Fed. Cir. 1995) (Testing for the full safety and effectiveness ... is more properly left to the Food and Drug Administration (FDA). Title 35 does not demand that such human testing occur within the confines of Patent and Trademark Office (PTO) proceedings).

Applicant also respectfully points the Examiner to the decision in *In re Bundy* (a copy enclosed) wherein the United States Court of Customs and Patent Appeals held that all that is necessary to satisfy the how-to-use (*i.e.*, enablement) requirement of 35 U.S.C. § 112 is the disclosure of some activity coupled with the knowledge as to the use of this activity. *In re Bundy*, 642 F.2d 430, 434 (C.C.P.A. 1981). The Court found that the claims were enabled notwithstanding the absence of examples of dosages for human use or animal tests. In explaining its reasoning, the Court stated that the early filing of an application with its disclosure of novel compounds which possess significant therapeutic use is to be encouraged. *Id.* The Court further stated that specific testing of thousands of compounds...in order to satisfy 35 U.S.C. § 112 would delay disclosure and frustrate, rather than further, the interests of the public. *Id.* Furthermore, the Court noted that one skilled in the art would know how to use the compounds to determine the specific dosages for the various biological purposes. *Id.*

Applicant respectfully submits that the present claims are enabled because the specification demonstrates an activity (*i.e.*, JNK inhibition) of the compounds and a use (*i.e.*, treatment of claimed diseases) for compounds having the activity. In addition to the references provided in Applicant's prior response which demonstrate the nexus between JNK inhibition and claimed diseases, Applicant submits herewith an additional peer-reviewed publication which points to kinase inhibitors which are currently in clinical trials. *See* page 1198 of Force *et al.*, "Inhibitors of Protein Kinase Signaling Pathways - Emerging Therapies for Cardiovascular Disease," *Circulation* 109:1196-1205 (2004) ("Force"), a copy enclosed. Applicant respectfully submits that Force rebuts any doubt one skilled in the art might have regarding the use of kinase inhibitors to treat diseases associated with kinases *in vivo*. Applicant notes the wide range of diseases represented by these clinical trials *viz.* cancer (*e.g.*, chronic myelogenous leukemia and lung cancer), diabetes, diabetic neuropathy, neurodegeneration, inflammation, rheumatoid arthritis, acute coronary syndrome, Crohn's disease, stroke and immunosuppressant therapy.

In addition, Applicant further notes that Force confirms that kinase signaling pathways underlie the molecular basis of cardiac hypertrophy, rebutting any doubt raised by Yano *et al.* See Abstract of Force.

The Examiner has stated that the claims are directed to unrelated conditions, that there are potentially many different etiologies, and that each of the claimed diseases may or may not be addressed by mechanisms involving the JNK pathway. Applicant respectfully disagrees, and submits that the pending claims are directed only to certain diseases responsive to inhibition of the JNK pathway (*i.e.*, claim 1) and certain ischemic diseases (*i.e.*, claims 17 and 27). In other words, contrary to the Examiner's assertion, the claims are not directed to unrelated conditions or diseases having different etiologies and to every inflammatory disease or every cancer. Rather, the claims are specifically directed to specific ischemic diseases, which Bennett *et al.* teaches are associated with JNK, and to specific conditions which are treatable by inhibition of the JNK pathway.

In summary, Applicant respectfully submits that the present claims are enabled by the specification because compounds of the claims are shown to be JNK inhibitors and the scientific literature abundantly points to the nexus between inhibition of the JNK pathway and the treatment of the diseases recited in the claims. Accordingly, Applicant submits that claims 1, 17 and 27-33 satisfy the enablement requirement of 35 U.S.C. §112, first paragraph.

Claims 34-45 have been canceled without prejudice.

In view of the above remarks, it is believed that the rejection of claims 1, 17 and 27-45 under 35 U.S.C. §112, first paragraph, has been overcome and should be withdrawn.

Conclusion

Applicant respectfully requests that the present amendments be entered and the present remarks be made of record in the file history of the present application. An early allowance of the application is earnestly requested. The Examiner is invited to call the undersigned with any questions concerning the foregoing.

No fees other than those due in connection with the filing of the Terminal Disclaimer and the Petition for Extension of Time are believed to be due in connection with this response; however, should any other fee be required, Applicant hereby authorizes that the required fee be charged to Jones Day Deposit Account No. 50-3013.

Date: February 13, 2006

Respectfully submitted,
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ing said wall portions of each compartment to define a space having a thickness equal to a second predetermined distance approximately equal to the thickness of the first enclosure for guiding the first enclosures into said compartments and preventing the first enclosures from moving therein, an elongate rectangular recess provided in at least one of said spaced parallel wall portions of each compartment which opens at said compartment open end and is spaced from said further wall portions so as to form a depth limiting shoulder adapted to maintain at least a portion of said second enclosure projecting above said parallel wall portions upon full insertion of said second enclosure into said compartment, each recess forming retaining wall portions which define a space having a width greater than said first predetermined distance and a thickness smaller than said second predetermined distance suitable for securely receiving a second enclosure to thereby prevent movements of a second enclosure within said compartments whereby the manual gripping of any second enclosure is facilitated by said depth limiting shoulder, and whereby each compartment can interchangeably receive either a first or a second enclosure and maintain each securely therein by insertion through an associated compartment open end and respective cooperation with either said guide means or said retaining wall portions of said recesses.

Nowhere in the design applications is the word "insert" used, nor is there any indication that the interiors of the cases are inserts. The drawings do not disclose how the insert can be used to accommodate either cassette or cartridge type tape enclosures. Berkman argues that one skilled in the art would readily recognize that the interiors of the cases illustrated in the design drawings are inserts. We do not agree. There is nothing shown in the drawings to lead one of ordinary skill to such a conclusion.

Because Berkman's design applications fail to disclose the claimed invention sufficiently to comply with the requirements of

§ 112 first paragraph, his utility application is not entitled under 35 U.S.C. § 120 to the benefit of the filing date of the design applications. The § 102(b) rejection is affirmed.

AFFIRMED.



In re Gordon L. BUNDY

Appeal No. 80-591.

United States Court of Customs and
Patent Appeals.

Feb. 26, 1981.

Appeal was taken from decision of the trademark trial and appeal board affirming rejection of sole claim of patent application, Serial No. 832,329. The Court of Customs and Patent Appeals, Nies, J., held that: (1) the how-to-use requirement of patent statute was satisfied by disclosure of utility of invention, which related to new series of analogs of naturally-occurring prostaglandins, in terms of being useful and used in same manner as known E-type prostaglandins, and (2) the court could infer no withholding of information as to best mode of use from applicant's general statements of increased selectivity and narrower spectrum of potency for the novel analogs, conclusions which could be drawn from elementary pharmacological testing of analogs which established basic E-type activity.

Reversed.

1. Patents ⇐ 113(6), 114.19

The Patent and Trademark Office must have adequate support for its challenge to credibility of applicant's statements as to utility; only then does burden shift to applicant to provide rebuttal evidence. 35 U.S.C.A. § 112.

2. Patents \Rightarrow 99

The how-to-use requirement of patent statute was satisfied by disclosure of utility of invention, which related to new series of analogs of naturally-occurring prostaglandins, in terms of being useful and used in same manner as known E-type prostaglandins. 35 U.S.C.A. § 112.

3. Patents \Rightarrow 98

In patent case, the district court could infer no withholding of information as to best mode of use from applicant's general statements of increased selectivity and narrower spectrum of potency for its novel analogs, conclusions which could be drawn from elementary pharmacological testing of analogs which established the basic E-type activity. 35 U.S.C.A. § 112.

4. Patents \Rightarrow 99

Satisfaction of best mode requirement of patent statute is a question separate and distinct from question of sufficiency of disclosure to comply with enablement provision. 35 U.S.C.A. § 112.

5. Patents \Rightarrow 99

Best mode requirement of patent statute does not require one to obtain further knowledge but only to disclose what one knows or, at least, contemplates. 35 U.S.C.A. § 112.

Robert A. Armitage, Kalamazoo, Mich., for appellant.

1. Serial No. 832,329, filed September 12, 1977, for 3,7-Inter-m-Phenylene-4,5,6-Trinor-2-Decarboxy-2-Hydroxymethyl-9-Deoxy-9-Methylene-PGF-Type Compounds. The application is a divisional application of Ser. No. 682,848, filed May 4, 1976, issued as U. S. Patent No. 4,060,534 on November 29, 1977.

2. The first paragraph of § 112 reads:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Natural prostaglandins are found in mammalian tissues and have varied pharmacologic

Joseph F. Nakamura, Sol., for Patent & Trademark Office; Gerald H. Bjorge, Washington, D. C., of counsel.

Before MARKEY, Chief Judge, and RICH, BALDWIN, MILLER and NIES, Judges.

NIES, Judge.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Appeals (board) affirming the rejection of the sole claim of appellant's application¹ under the first paragraph of 35 U.S.C. § 112.² We reverse.

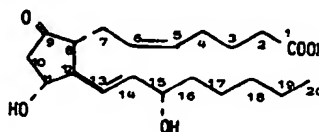
The appeal raises questions regarding the extent to which new pharmaceuticals must be tested, preceding the filing of an application, in order to satisfy the how-to-use and best mode requirements of § 112.

The Invention

The invention relates to a new series of analogs of naturally-occurring prostaglandins³ which differ from the corresponding known prostaglandins in that these analogs have a methylene group at the C-9 position.⁴ Structurally, the compounds may be considered analogs of either E-type prostaglandins (PGEs) in which the methylene group replaces the usual C-9 keto- or oxo-group or of F-type prostaglandins (PGFs) in which the methylene group replaces the

uses including the treatment of hypertension, ulcers and asthma, and the interruption of pregnancy. In naming the prostaglandins, the prefix PG is followed by a letter designating the oxidation state of the cyclopentane ring; thus arise the series PGA, PGE, PGF, etc. The numeral subscript refers to the number of double bonds in the side chain. 1 D. Lednicher & L. Mitscher, *The Organic Chemistry of Drug Synthesis*, 23-27 (1977).

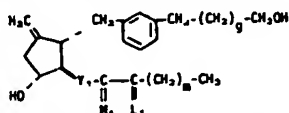
4. A typical example of a naturally-occurring prostaglandin is PGE₂ which structurally is represented:



C-9 hydroxyl group. Pharmacologically, however, the analogs are related only to PGEs.

The sole claim reads:

131. A prostaglandin analog of the formula



wherein Y_1 is trans-CH=CH- , $\text{-C}\equiv\text{C-}$, or $\text{-CH}_2\text{CH}_2\text{-}$;

wherein M_1 is



or



wherein R_3 is hydrogen or methyl;

wherein L_1 is



or a mixture of



and



wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro;

wherein g is one, 2 or 3; and

wherein m is one to 5, inclusive.

The Disclosure

The specification of U. S. Patent No. 4,060,534 ('534) has been incorporated by reference to serve as the specification for the present application. The portions of the specification directed to using these novel analogs are pertinent to the issues on appeal.

The background section of the specification contains a detailed description of the uses of various *known* PGEs. Nine specific biological responses caused by PGEs, ranging from decreasing blood pressure to inhibiting gastric secretion, are listed. Based on these responses, various pharmacological uses with broad ranges of dosage by various methods of administration are enumerated.

The use of appellant's novel analogs, which include not only the claimed compounds of this application, but also those claimed in other divisional applications and in '534, is subsequently set forth:

The novel prostaglandin analogs of this invention correspond to the prostaglandins described above in that the novel prostaglandin analogs exhibit prostaglandin-like activity.

Specifically the 9-deoxy-9-methylene-PGF-type compounds of this invention correspond to the PGE compounds described above, in that these novel 9-deoxy-9-methylene-PGF-type compounds are useful for each of the above-described purposes for which the PGE compounds are used, and are used in the same manner as the PGE compounds, as described above.

The PGE compounds described above, are all potent in causing multiple biological responses even at low doses. Moreover, for many applications, these prostaglandins have an inconveniently short duration of biological activity. In striking contrast, the novel prostaglandin analogs of this invention are substantially more selective with regard to potency in causing prostaglandin-like biological responses, and have a substantially longer duration of biological activity. Accordingly, each of these novel prostaglandin analogs is surprisingly and unexpectedly more useful than one of the corresponding prostaglandins described above for at least one of the pharmacological purposes indicated above for the latter, because it has a different and narrower spectrum of biological potency than the known prostaglandin, and therefore is more specific in its activity and causes smaller and fewer undesired side effects than when the prostaglandin is used for the same purpose. Moreover, because of its prolonged activity, fewer and smaller doses of the novel prostaglandin analog are frequently effective in attaining the desired result.

The specification includes a disclosure relating to preparation of the compounds generally, and several specific examples. None, however, are compounds within the subgenus claimed in this application.

No example of a specific use of any of the disclosed prostaglandin analogs, i. e., setting forth a dosage to achieve a desired response, is given.

The Rejection

The examiner rejected the sole claim under the first paragraph of 35 U.S.C. § 112

as being "inadequately supported by the instant specification" in that not a single example was directed to one of the claimed compounds. Failure to meet the best mode requirement was also raised on the basis of no exemplification. Reliance on utilities similar to known PGEs was attacked on the basis of a statement in a "Samuelsson et al. reference" (more correctly, a Rosenthale paper therein)⁵ that "small changes in the [prostaglandin] molecule can alter potency or even induce diametrically opposite pharmacologic effects." Thus, the utilities asserted on the basis of those known for structurally analogous compounds were said to be "at best highly speculative."

Before the board the § 112 rejection was more specifically explained by the examiner to encompass an inadequate disclosure of: (1) the description of the compounds; (2) the preparation of the same; (3) their use; and (4) the best mode of carrying out the invention. The examiner added that an undue amount of experimentation would be required to prepare the claimed compounds and to determine their utilities.

The board held that the description and how-to-make requirements of the first paragraph of 35 U.S.C. § 112 were satisfied by appellant's disclosure. It agreed with the examiner, however, that:

[U]ndue experimentation would be required on the part of one of ordinary skill in the relevant art to determine how to use the compounds claimed. Since we consider the manner of using a compound to be necessarily a part of "the best mode contemplated by the inventor of carrying out the invention", we also agree with the examiner's position that the best mode requirement has not been met.

The challenge raised by the examiner's citation of the Rosenthale paper was deemed reasonable and un rebutted by any factual evidence. The board then added:

5. Cited by the examiner as: Samuelsson et al., *Advances in Prostaglandin and Thromboxane Research*, Vol. 1 (1976) 488-491.

Appellant has pointed out that the work relied upon is a paper by Rosenthale et al. entitled

[O]ne of the advantages alleged for the compounds here claimed is that they are more selective than the analogous PGE compounds. This is an express indication that not all of the compounds covered by appellant's claims will induce the same biological responses.

Accordingly, the board affirmed the examiner's rejection of the sole claim to the extent it was based on the how-to-use and best mode requirements of § 112.

OPINION

How-to-Use

The enablement question present here is whether the disclosure of utility in terms of being useful and used in the same manner as known PGEs is sufficient to satisfy the how-to-use requirement of the first paragraph of 35 U.S.C. § 112.

[1, 2] The PTO must have adequate support for its challenge to the credibility of applicant's statements as to utility. Only then does the burden shift to appellant to provide rebuttal evidence. *In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA 1973); *In re Marzocchi*, 58 CCPA 1069, 439 F.2d 220, 169 USPQ 367 (1971). We must consider the Rosenthale paper in its entirety in determining the reasonableness of the doubt raised by the authors' conclusory statement relied on by the examiner, and in so doing see no specific evidence that structural variations of PGEs cause opposite pharmacologic effects. The tests reported by Rosenthale do indicate shifts in PGF_{2α} activity from bronchoconstrictor to bronchodilator concomitant with structural changes. For PGEs Rosenthale shows only variations in potency, a matter of degree of activity. Accordingly, we do not agree that Rosenthale is sufficient support for the examiner's position that the subject analogs, related as they are to PGEs in pharmacological activity, may not be useful at all to achieve a particular response.

titled "Actions of Prostaglandins on the Respiratory Tract of Animals," pp. 477-493 included in the above book, edited by Samuelsson et al. Henceforth we shall refer to this reference as the Rosenthale paper.

The board focused on another reason for challenging the disclosure as non-enabling. Appellant's disclosure of increased "selectivity" of the novel analogs was taken as an express indication that it was uncertain "which compound will induce which biological responses . . .," thus virtually ensuring that an undue amount of experimentation would be required to use the invention. The ranges of dosage for known PGEs, assuming their applicability to appellant's analogs, were said to be very broad and would, in any event, provide little guidance in determining dosages for the more selectively functional claimed analogs.

Appellant contends that the disclosure teaches that *all* novel compounds exhibit *each* of the enumerated pharmacological uses. The increased selectivity is said to be with respect to the potency for each activity, not to the existence of that biological activity. Any contrary interpretation of the specification is strongly denied. As far as determining dosages for the novel analogs is concerned, it is urged that the experimentation needed to ascertain proper levels for various responses would not be undue, but rather would lie well within the ability of one of ordinary skill in the art. At most, appellant states, the question is whether the determinations would be extended, not undue.

We have no difficulty with appellant's interpretation of "selectivity". In the pertinent section, previously quoted, it is clearly stated that the novel compounds are "useful for *each* of the above-described purposes for which the PGE compounds are used" (emphasis added). This can only reasonably be read as teaching that *each* compound can be used for *each* and every one of the aforesaid biological responses. Appellant's further statements that the novel analogs are "substantially more selective with regard to potency" or "more specific in its activity" because of a "different and narrower spectrum of biological potency," does not negate the asserted usefulness for each purpose. There is no requirement that all have the same degree of activity for each use. What is necessary to satisfy the how-to-use requirement of § 112 is the dis-

closure of some activity coupled with knowledge as to the use of this activity. *In re Gardner*, 475 F.2d at 1392, 177 USPQ at 398.

Thus the remaining question is whether appellant's disclosure is sufficient to enable one of ordinary skill in the art to use these novel analogs. No specific examples of dosages for human use or even animal tests are given for the novel compounds *per se*. Appellant's counsel stated at oral argument that all that had been established at the time of filing the application was the basic pharmacology for these compounds. Appellant's specification discloses that these compounds possess activity similar to E-type prostaglandins. As to the latter, dosages are disclosed, albeit expressed in very broad ranges.

We do not consider that one of ordinary skill in the art would not know how to use these novel analogs to determine the specific dosages for the various biological purposes. We are persuaded that sufficient guidelines as to use are given in the disclosure here. This is not the same situation as in *In re Gardner et al.*, 57 CCPA 1207, 427 F.2d 786, 166 USPQ 138 (1970). Here only the compounds themselves are being claimed, not their therapeutic use. Nor can a parallel be drawn to *In re Kirk*, 54 CCPA 1119, 376 F.2d 936, 153 USPQ 48 (1967), the basic pharmacological activity having been established in this case, not merely *presumed* from similar molecular structure.

Early filing of an application with its disclosure of novel compounds which possess significant therapeutic use is to be encouraged. Requiring specific testing of the thousands of prostaglandin analogs encompassed by the present claim in order to satisfy the how-to-use requirement of § 112 would delay disclosure and frustrate, rather than further, the interests of the public.

Accordingly, we are satisfied that the how-to-use requirement of the first paragraph of § 112 has been adequately complied with by appellant's disclosures.

Best Mode

[3] Turning to the best mode issue, we agree with appellant that this rejection was

founded on a lack of enablement by both the examiner and the board. Our holding that appellant has adequately told how to use the novel compounds necessarily undercuts this position. However, we do not agree that the thrust of the inquiry is the same for determining satisfaction of the further requirement that the specification shall set forth the best mode contemplated by the inventor of carrying out his invention.

[4, 5] Satisfaction of the best mode requirement of § 112 is a question separate and distinct from the question of the sufficiency of the disclosure to comply with the enablement provision. *In re Gay*, 50 CCPA 725, 731, 309 F.2d 769, 772, 135 USPQ 311, 315 (1962). The question is one of concealment, i. e., whether an applicant has withheld what he considers to be the best mode of carrying out his invention. The best mode requirement does not require one to obtain further knowledge but only to disclose what one knows or, at least, contemplates.

The Solicitor argued that concealment may be inferred. Quoting the disclosure in the specification that each analog is "surprisingly and unexpectedly more useful than one of the corresponding prostaglandins . . . for at least one of the pharmacological purposes . . .," he urges that appellant must have had test results to substantiate this statement and this data should have been disclosed. The alleged withholding of information on which these general statements were made is said to render the quality of disclosure so poor that it effectively results in concealment, citing *In re Sherwood*, 613 F.2d 809, 816, 204 USPQ 537, 544 (CCPA 1980).

Were we to see merit in the Solicitor's position fairness would require providing appellant with the opportunity to present evidence in rebuttal. However, we do not find it necessary for appellant to assume this burden of proof. We can infer no withholding of information as to the best mode of use from appellant's general statements of increased selectivity and narrower spectrum of potency for these novel ana-

logs, conclusions which could be drawn from the elementary pharmacological testing of the analogs which established the basic E-type activity.

Accordingly, we reverse the holding that the best mode requirement has not been satisfied.

CONCLUSION

The board's affirmance of the rejection of appellant's sole claim under both the how-to-use and the best mode requirements of the first paragraph of § 112 is reversed.

REVERSED.



The UNITED STATES, Appellant,

v.

SANYO ELECTRIC INC., Appellee.

No. 80-34.

United States Court of Customs
and Patent Appeals.


March 5, 1981.

Importer brought suit protesting Government's classification and hence, rate of duty assessed, for merchandise invoiced as power failure lights. The Customs Court, now the United States Court of International Trade, Re, C. J., 496 F.Supp. 1311, found Government's classification was erroneous and classified light with duty at rate of 5.5 percentum ad valorem, and United States appealed. The Court of Customs and Patent Appeals, Baldwin, J., held that imported power failure lights were incorrectly classified as "flashlights" and should have been classified as "electrical articles * * * not specifically provided for."

Order accordingly.

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Inhibitors of Protein Kinase Signaling Pathways: Emerging Therapies for Cardiovascular Disease

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Inhibitors of Protein Kinase Signaling Pathways Emerging Therapies for Cardiovascular Disease

Thomas Force, MD; Keisuke Kuida, MD; Mark Namchuk, PhD;
Keykavous Parang, PhD; John M. Kyriakis, PhD

Abstract—Protein kinases are enzymes that covalently modify proteins by attaching phosphate groups (from ATP) to serine, threonine, and/or tyrosine residues. In so doing, the functional properties of the protein kinase's substrates are modified. Protein kinases transduce signals from the cell membrane into the interior of the cell. Such signals include not only those arising from ligand–receptor interactions but also environmental perturbations such as when the membrane undergoes mechanical deformation (ie, cell stretch or shear stress). Ultimately, the activation of signaling pathways that use protein kinases often culminates in the reprogramming of gene expression through the direct regulation of transcription factors or through the regulation of mRNA stability or protein translation. Protein kinases regulate most aspects of normal cellular function. The pathophysiological dysfunction of protein kinase signaling pathways underlies the molecular basis of many cancers and of several manifestations of cardiovascular disease, such as hypertrophy and other types of left ventricular remodeling, ischemia/reperfusion injury, angiogenesis, and atherogenesis. Given their roles in such a wide variety of disease states, protein kinases are rapidly becoming extremely attractive targets for drug discovery, probably second only to heterotrimeric G protein–coupled receptors (eg, angiotensin II). Here, we will review the reasons for this explosion in interest in inhibitors of protein kinases and will describe the process of identifying novel drugs directed against kinases. We will specifically focus on disease states for which drug development has proceeded to the point of clinical or advanced preclinical studies. (*Circulation*. 2004;109:1196-1205.)

Key Words: drugs ■ kinases ■ pharmacology ■ inhibitors

A consensus is emerging that protein kinase modulators will be effective treatments for a variety of diseases.¹ However, protein kinases were initially thought to be unsuitable drug targets, in large part because of what was perceived to be an unfavorably high degree of structural conservation within key domains of all protein kinases. Because binding of ATP to kinases is essential for kinase activity and properties of the protein kinase ATP-binding pocket were well understood, agents targeting the ATP pocket were the logical first choice for drug development. However, the structural conservation of protein kinase ATP binding sites and the presence of more than 500 protein kinases in the human genome² led to the belief that highly selective small-molecule protein kinase inhibitors targeting the ATP pocket would be difficult to generate. As will be discussed below, the development and characterization of inhibitors of the p38 mitogen-activated protein kinases (MAPKs) indicated that this initial belief was misguided. A second argument against targeting protein kinases for drug development was the observation that modulation of a protein kinase could in one system prove

beneficial, while proving deleterious in another. As an extreme example of this, inhibiting a protein kinase required for triggering programmed cell death could reduce ischemia-induced cell death in terminally differentiated cardiomyocytes but might also favor tumor promotion in other organs or cell types. Finally, toxicity with long-term use was a concern. Thus, inhibiting a protein kinase that is dysregulated in one organ in a particular disease state may prove harmful to other systems in which that same protein kinase is not dysregulated but instead serves essential functions. For example, inhibiting the cell-surface HER2 tyrosine kinase receptor with the monoclonal antibody trastuzumab (Herceptin, Genentech) in patients with breast cancers overexpressing that receptor has produced strikingly beneficial results, but it has come at the expense of severe cardiac dysfunction in some women receiving the therapy, suggesting a critical role for this receptor in cardiomyocyte survival.³

All of the above concerns being noted, the “proof of principle” of the tremendous therapeutic potential of small-molecule inhibitors of protein kinases came with the discov-

From the Molecular Cardiology Research Institute, Tufts-New England Medical Center and Tufts University School of Medicine, Boston, Mass (T.F., J.M.K.); Vertex Pharmaceuticals, Inc, Cambridge, Mass (K.K., M.N.); and the Department of Biomedical Sciences, University of Rhode Island, Kingston (K.P.).

Drs Namchuk and Kuida are employees of and Dr Force receives financial support for his laboratory from Vertex Pharmaceuticals, Inc, which produces small-molecule inhibitors of protein kinases, are the subject of this article.

Additional material may be found in the Data Supplement with the online-only version of this article at <http://www.circulationaha.org>.

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ery of imatinib mesylate (Gleevec, STI-571, Novartis), an ATP-competitive small-molecule inhibitor of the tumorigenic fusion protein Bcr-Abl (reviewed by Barnes and Melo⁴) (Table; Figure 1). c-Abl is a nuclear protein tyrosine kinase the biological function of which is unclear (although it may function in sensing the integrity of the genome and promoting programmed cell death). Bcr is a multifunctional cytosolic polypeptide that may play a role in regulating activity of the Rho subfamily of small G proteins. The fusion of Bcr and Abl to produce Bcr-Abl arises from the chromosomal translocation that creates the Philadelphia chromosome. Unlike c-Abl, Bcr-Abl is both cytosolic and nuclear, and because it forms homodimers that cross-phosphorylate and activate one another, Bcr-Abl manifests constitutively active and inappropriately directed Tyr kinase activity. Bcr-Abl is causal in chronic myelogenous leukemia, and treatment with imatinib has been able to induce complete remissions, at least in the early stages of the disease.⁴

Indeed, the cancer field has led the way in spurring on drug development directed both at protein kinases that, like Bcr-Abl, are activated by mutations and lead directly to growth deregulation and at "permissive" protein kinases that, while otherwise normal themselves, serve as essential effectors for mutant, deregulated gene products. The protein kinases MAPK ERK kinase (MEK)1/2, which activate the extracellular signal-regulated kinase (ERK) family of MAPKs (Figure 2), and the mammalian target of rapamycin (mTOR) are 2 such permissive kinases that play roles in cell cycle progression. Inhibitors of these kinases (U0126 and PD184352 [Figure 1] and rapamycin/sirolimus, respectively) are in clinical trials for the treatment of a variety of tumors (Table). In addition, rapamycin/sirolimus is currently used with dramatic success as an immunosuppressant and an inhibitor of in-stent restenosis.⁵ Early successes with agents targeting protein kinases have led to the logical conclusion that in the future, cancers will be defined not only by tumor type and stage but also by the protein kinase activity profile (ie, which kinases are dysregulated).⁶ It is likely that the same will be true for complex disease states of the cardiovascular system.

Developing an Inhibitor

A major issue in drug development is the identification of appropriate targets for therapeutic intervention. To identify a protein kinase as a putative therapeutic target, it is not sufficient simply to know whether it is activated (or inhibited) in a specific disease state, because dysregulation can be an irrelevant consequence of the disease rather than a key contributing factor to disease pathology. At the very least, clear genetic or physiological/cell biological data are needed that implicate a protein kinase as an attractive target.

Once a kinase is validated as a potential target for drug development, screening of chemical libraries is performed to identify possible inhibitors. Many large pharmaceutical companies possess enormous chemical libraries consisting of hundreds of thousands of synthetic compounds. The identification of one or more of these as a candidate inhibitor requires a process called high-throughput screening (HTS). (For the interested reader, a more detailed description of the

process of HTS is available on-line and in Reference 7.) A good, robust, and reliable HTS assay can be used to screen >100 000 small molecules in a day. Typical "hit rates" for an unbiased screen might be only 0.1% to 0.3%; therefore, various strategies have been devised to improve hit rates by focusing the screen. Focusing of the library of compounds can be based on the actual crystal structure of the ATP-binding pocket of the kinase or a family member if known (structure-based library design) or on the structure of compounds already known to bind to the ATP pocket if available (ligand-based library design). These virtual screening or molecular modeling approaches to screen more targeted libraries not only can improve the hit rate but also may reduce the duration and expense of primary screens.⁷

Binding of ATP to a protein kinase is essential for the kinase's phosphotransferase activity, and thus, the ATP-binding pocket is the "target" of most inhibitor screens. As was noted above, this idea initially seemed counterintuitive, given the structural conservation of protein kinase ATP-binding sites.⁸ However, there is, in fact, enough structural diversity in these sites⁸ to predict that selective ATP-competitive inhibitors can be identified. Indeed, contrary to initial concerns, screens of unbiased compound libraries have identified several ATP competitors that function as relatively selective inhibitors.^{9,10}

For a protein kinase inhibitor to have a chance of clinical efficacy, it must bind to the target kinase with an extremely high affinity: several orders of magnitude higher than that of ATP, because the inhibitor will be present in concentrations typically in the mid to high nanomolar range, whereas the intracellular concentration of ATP is millimolar. This suggests that any initial "hits" from an HTS will most likely benefit from optimization to improve potency and selectivity. The efficiency of the optimization process is greatly augmented by the abundant x-ray crystallographic information available for kinase families. Thus, the structure-activity relationship of any compound can be correlated with specific molecular interactions of the compound with the kinase active site, and in this way, the structure of the inhibitor can be optimized.⁷ When no structure data exist for a specific kinase, knowledge of the structure of another member of the family can often be used to create binding models from which optimized compounds can be synthesized.⁷

The need for an extremely high binding affinity of an inhibitor to the ATP pocket and the relative similarities of ATP pockets across protein kinase families suggest that it may be beneficial to examine protein kinases for determinants in addition to the ATP pocket that might confer additional specificity. Here, the MAPKs provide an excellent example. The ability of different MAPK groups to interact with and then phosphorylate selective intracellular protein substrates is conferred by a specific substrate docking site of the MAPKs, the common docking (CD) domain, that is quite distal from the ATP binding site.¹¹ The CD motifs of MAPKs bind complementary sites on the corresponding MAPK substrates (and on MAPK regulators) (eg, MEK1 binding to ERK-1 [Figure 2] is mediated by the CD domain). Although there is substantial sequence conservation among MAPK CD domains, the sequence divergence is sufficient to enable

Selected Inhibitors of Protein Kinases in Clinical Trials

Kinase Target	Agent	Trial (Disease)	Sponsor
Tyrosine kinases			
ABL (c-Kit, PDGFR)	Gleevec (STI-571)	Approved (CML)	Novartis
EGFR	ZD1839 (Iressa)	Approved (lung cancer)	AstraZeneca
	OSI-774	Phase III (cancer)	OSI/Roche/Genentech
	IMC-C225 (mAb)	Phase III (cancer)	ImClone
	ABX-EGF (mAb)	Phase II (cancer)	Abgenix
	MDX-447 (mAb)	Phase I (cancer)	Merck KgaA
	EMD 72000 (mAb)	Phase I (cancer)	Merck KgaA
	Genistein	Phase II (cancer)	NCI
	RH3 (mAb)	Phase II (cancer)	York Medical Bioscience Inc
EGFR, ERB2R	CI1033	Phase II (cancer)	Pfizer
	EKB569	Phase I (cancer)	Wyeth-Ayerst
	GW2016	Phase I (cancer)	GlaxoSmithKline
	PK1166	Phase I (cancer)	Novartis
VEGFR (PDGFR, FGFR)	SU6668	Phase I (cancer)	Pharmacia Corp
PDGFR (Ft-3)	CT53518	Phase I (cancer)	Millennium Pharmaceuticals
VEGFR	SU5416	Phase III (cancer)	Pharmacia Corp
	PTK787/ZK222584	Phase II (cancer)	Novartis/Schering-Plough
VEGFR (EGFR)	ZD6474	Phase II (cancer)	AstraZeneca
VEGFR (PDGFR)	SU011248	Phase II (cancer)	Sugen
NGFR, Trk	CEP-2583	Phase II (cancer)	Cephalon
HER-2/neu	17-AAG	Phase I (cancer)	Kosan
	Trastuzumab (mAb)	Approved (cancer)	Genetech
	2C4 (mAb)	Phase I (cancer)	Genetech
	CP-724,714	Phase I (cancer)	OSI Pharmaceuticals/Pfizer
	MDX-210 (mAb)	Phase I (cancer)	Novartis
Serine/threonine kinases			
PKC, c-Kit, PDGFR	PKC412	Phase II (cancer, retinopathy)	Novartis
PKC	ISIS 3521	Phase III (cancer)	ISIS Pharmaceuticals
	CGP41251	Phase II (cancer)	Novartis
	UCN-01	Phase I/II (cancer)	Kyowa Hakko Kogyo
	Bryostatins-1	Phase I/II (cancer)	Biotek
PKC- β	Ly333531	Phase I (cancer)	Eli Lilly
		Phase II/III (diabetic neuropathy)	
CDKs	Flavopiridol	Phase II (cancer)	Aventis
	E7070	Phase I (cancer)	EISAI
	BMS-387032	Phase I (cancer)	Bristol-Myers Squibb
	CYC202	Phase I (cancer)	Cyclacel
MEK1/2	PD184352	Phase II (cancer)	Pfizer
	U-0126	Phase I (cancer)	Promega
MLK	CEP-1347	Phase II (neurodegeneration)	Cephalon
RAF	BAY43-9006	Phase II (cancer)	Onyx Pharmaceuticals/Bayer
	ISIS5132	Phase II (cancer)	Isis pharmaceuticals
	L-779,450	Phase II (cancer)	Merck
Ras	ISIS2503	Phase II (cancer)	Isis pharmaceuticals
	SCH66336	Phase II (cancer)	Schering-Plough
	BMS214662	Phase I (cancer)	Bristol-Myers Squibb
	R115777	Phase I/II (cancer)	Johnson & Johnson
mTOR	CCI779	Phase II (cancer)	Wyeth-Ayerst
	RAD001	Phase I (cancer)	Novartis
		Phase II/III (immunosuppressant)	
	Rapamycin	Approved (immunosuppressant)	Wyeth-Ayerst
p38-MAPK	VX702	Phase II (inflammation; ACS)	Vertex Pharmaceuticals
	BIRB796	Phase III (inflammation; RA; Crohn's)	Boehringer Ingelheim
	SCIO-323	Phase I (RA; stroke; diabetes)	Scios, Inc
	SCIO-469	Phase II (RA; Crohn's)	Scios, Inc
PDK1	UCN-01	Phase I/II (cancer)	Kyowa Hakko Kogyo
JNK1-3	CC401	Phase I	Celgene

VEGFR indicates vascular endothelial growth factor receptor; PDGFR, PDGF receptor; FGFR, fibroblast growth factor receptor; CML, chronic myelogenous leukemia; RA, rheumatoid arthritis; and ACS, acute coronary syndromes. Inhibitors are of two types, monoclonal antibodies (mAbs), which are directed at the extracellular domain of various receptor tyrosine kinases, and small-molecule inhibitors.

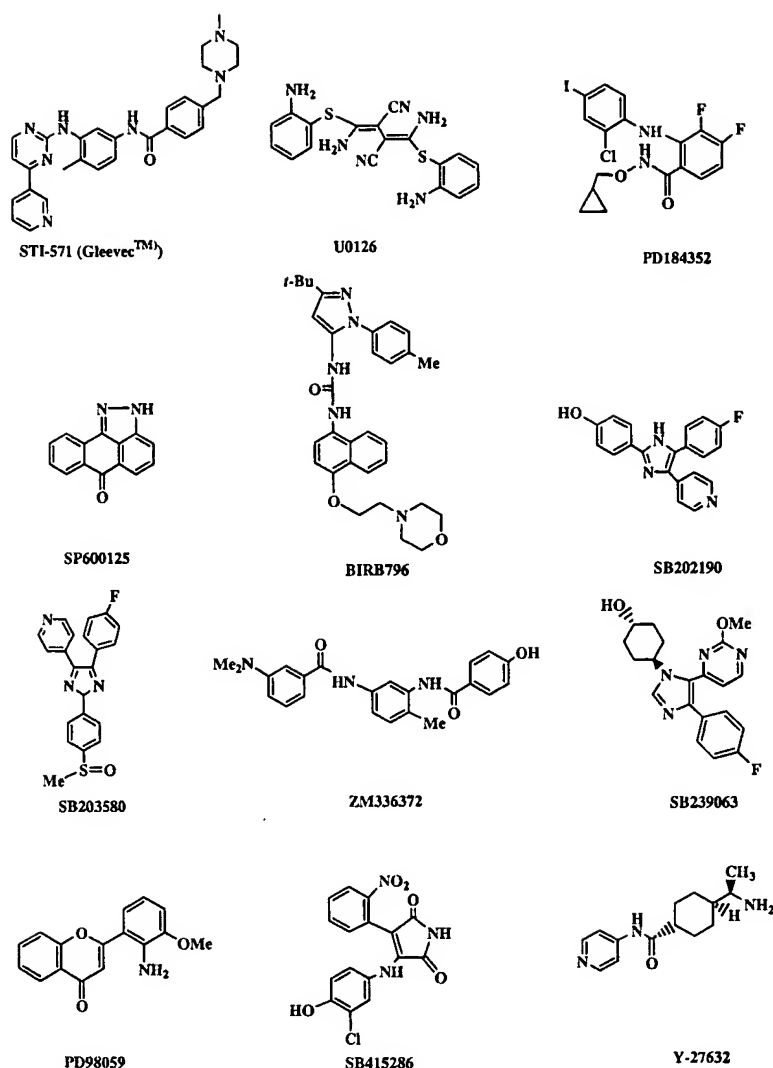


Figure 1. Chemical structures of several small-molecule protein kinase inhibitors referred to in text. These can be divided into inhibitors that are ATP-competitive, including phenylamino pyrimidines (eg, STI-571), pyridinylimidazoles (SB202190, SB203580, and SB239063), anthra-pyrazolones (SP600125), and maleimides (SB415286), and those that are non-ATP-competitive (MEK1/2 inhibitors, U0126, PD184352, and PD98059, which maintain kinases in an inactive state by preventing their phosphorylation by upstream activating kinases such as Raf). BIRB796, a pyrazole urea, is both noncompetitive and competitive (see text).

exquisite MAPK specificity. Of note, the CD domains are quite small (≤ 18 amino acids), contain key acidic residues, and reside on an exposed surface in the MAPK structure, suggesting that these domains could be ideal targets for drug design.¹¹

The use of determinants in addition to the ATP pocket combined with optimization based on crystal structure was recently used to optimize the design of a p38-MAPK inhibitor. Crystallography demonstrated that this inhibitor did not target the ATP binding pocket but rather targeted a novel site in the kinase active site that is exposed after a large conformational change that accompanies binding of the inhibitor.¹² Crystallography allowed the compound to be modified to optimize binding to the novel site and also to establish binding in the ATP pocket. This gives the final compound, BIRB796 (Figures 1 and 3), which is currently in clinical trials for various inflammatory disorders (Table), a high degree of potency and selectivity.

Another approach to inhibit MAPK signaling that might reduce toxicity would be to target upstream activators of the MAPKs rather than the MAPKs.¹³ For example, c-Jun N-terminal kinase (JNKs) are activated by at least 12 different

MAPK kinase kinases (MAPKKKs) and 2 MAPK kinases (MAPKKs; see legend to Figure 2 for terminology). Because specific MAPKKKs and MAPKKs transduce the activation of JNKs in response to specific stimuli¹⁴ (eg, MAPKK7 but not MAPKK4 is necessary for JNK activation by tumor necrosis factor [TNF]- α), one could potentially target MAPKK7 specifically with an inhibitor in patients with inflammatory disorders. This would leave JNK activation by other stimuli acting via MAPKK4, and essential cellular functions regulated by JNKs, at least partially intact.

Potency and Selectivity

Potency and selectivity are critical issues for the eventual effectiveness and safety of any drug. Potency is expressed as the enzymatic IC_{50} (concentration of drug that inhibits enzyme activity by 50%). However, reported IC_{50} s must be interpreted with caution, because the IC_{50} determined for an ATP-competitive inhibitor will vary depending on the concentration of ATP used in the assay and on the K_m (the affinity of the kinase for ATP).¹⁰ This has been a source of significant confusion in the literature. For example, results from assays of the widely used anthrapyrazolone JNK inhib-

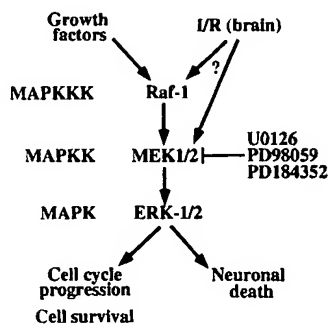


Figure 2. ERK cascade. All MAPKs described to date are part of a 3-tiered cascade whereby MAPKs, in this case, ERKs, are activated by upstream kinases (MAPKKs, in this case MEK1/2), which, in turn, are activated by a MAPKKK (in this case Raf-1). Growth factor-induced activation of pathway often leads to cell cycle progression and, in some cases, activation of survival pathways. I/R in brain also leads to ERK activation, but in this case it is deleterious, leading to neuronal death. It is not clear whether Raf-1 is the MAPKKK involved in I/R-induced activation of ERKs in brain. MEK inhibitors discussed in text are shown.

itor SP600125 (Figure 1) with 20 $\mu\text{mol/L}$ ATP initially suggested that SP600125 was a very potent inhibitor with a low IC_{50} . However, the results of studies that used assays with more “physiological” concentrations of ATP (100 $\mu\text{mol/L}$) recently demonstrated that SP600125 was, in fact, a relatively weak (and also nonselective) inhibitor with a high IC_{50} .¹⁰

Selectivity is a second key consideration in the design of kinase inhibitors. Compounds are “profiled” for their selectivity against panels of kinases (often 30 or more) to determine which targets, aside from the intended one, are being affected. These panels are chosen in a variety of ways but often include specific kinases that one does not want the drug to inhibit and/or a selection of kinases with a great deal of structural diversity at the active site (to broadly screen for nonspecific inhibition). Relative IC_{50} s of the drug for the

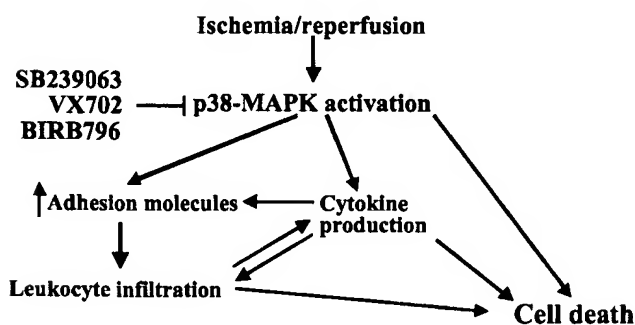


Figure 3. Mechanisms of p38-MAPK-induced cardiomyocyte death. I/R activates p38-MAPK, leading to both cytokine (and chemokine) production and upregulation of adhesion molecules on endothelial cells. This leads to leukocyte infiltration into ischemic region. Certain cytokines (eg, $\text{TNF-}\alpha$) are directly cytotoxic to cardiomyocytes. In addition, p38-MAPK probably also directly activates cell death pathways in ischemic cardiomyocytes (ie, cytokine- and leukocyte-independent effects on cell death). p38-MAPK inhibitors discussed in text are shown. Of note, JNKs also act to stabilize cytokine mRNA and, in addition, activate intrinsic cell death pathway by inducing release of cytochrome c from mitochondria.³⁶ This suggests that JNK inhibitors may also be protective against I/R injury (see text).

target kinase versus the others in the panel are then determined. Again, the concentration of ATP used in the assay is critical to allow an accurate comparison to be made. One approach to allow interpretation of relative IC_{50} s for an inhibitor between enzymes is to customize the assay conditions for the ATP affinity for each kinase in the screening panel (eg, fix the ATP concentration at the K_m for each kinase). Alternatively, others recommend using concentrations of ATP, $\geq 100 \mu\text{mol/L}$, that are well above the K_m for all of the kinases in the panel.¹⁰

What is an acceptable level of selectivity? There is no consensus, but in general, the goal is an IC_{50} that is at least 100-fold lower for the target kinase. However, this may vary depending on the indication, and in some cases, one might tolerate (or even prefer) agents that are not entirely selective. For example, in cancer, one might tolerate inhibition of kinases that positively regulate the cell cycle (cyclin-dependent kinases, Cdks) or that are antiapoptotic (eg, Akt) by a drug targeting Bcr-Abl, because antitumor activity might be greater. However, because of enhanced toxicity, one would not tolerate inhibition of Cdks or Akt by a drug targeting p38-MAPK for inflammatory diseases. Similarly, lack of selectivity for drugs that will be used short-term only might not be a major problem.

Once selectivity is determined in kinase assays *in vitro*, the selectivity profile is then determined in a cellular system. Given that cellular ATP concentrations are typically in the millimolar range, an upward shift in the cellular IC_{50} versus the enzymatic IC_{50} (performed at 100 $\mu\text{mol/L}$ or less) is often observed. The magnitude of this shift is dictated by a number of factors, including the ATP K_m for the target enzyme, the cellular permeability of the drug, and the amount of inhibition of the target kinase required to elicit the cellular response being monitored (eg, 20% inhibition of a particular kinase may be sufficient to lead to complete inhibition of a biological response). An effective general counterscreening strategy is to obtain enzymatic IC_{50} values for an extensive panel of biochemical kinase assays, then assess the cellular consequences of the observed inhibition pattern in cellular readouts biased to respond to inhibition of the signaling pathways represented in the enzymatic panel. If a drug with borderline selectivity in enzymatic assays has excellent characteristics in the cell-based assays (good inhibition of the target pathway, limited inhibition of other pathways, and no toxicity), the borderline enzymatic selectivity may be deemed adequate.

Finally, although the IC_{50} and selectivity studies (determined in assays *in vitro*) usually predict activity in the cell, this is not always the case. Thus, a compound with apparent high activity and specificity *in vitro* may display markedly different and even unexpectedly nonspecific activity *in vivo*. For example, the pyridinyl imidazoles SB203580 and SB202190 (Figure 1), which inhibit p38 MAPKs, are remarkably specific when assayed *in vitro* for inhibition of a variety of protein kinases.⁹ The basis for this specificity was revealed in the crystal structure of p38 α complexed with SB203580. To accommodate a fluorophenyl moiety present in the SB203580 structure, the amino acid at position 106 of the

kinase must be no larger than Thr.¹⁵ c-Raf, a protein kinase that activates the ERKs, is downstream of many growth factor receptors and plays a role in inducing cell-cycle progression (Figure 2), has a Thr (Thr321) at a site corresponding to Thr106 of p38 α . Not unexpectedly, therefore, c-Raf is inhibited by SB203580 and SB202092 *in vitro*, albeit at concentrations at least an order of magnitude higher than that needed to inhibit p38 α .^{13,14} However, in cell-based assays, the Raf-Mek-ERK pathway is not inhibited by SB203580 or SB202092. Surprisingly, SB203580 and SB202092 trigger a striking activation of c-Raf *in vivo*.¹⁶ Similarly, ZM336372 (Figure 1), a novel phenylamido derivative, is an *in vitro* Raf (and p38-MAPK) inhibitor but is a potent activator of c-Raf in intact cells. The basis for these paradoxical findings is unknown, but they are indicative of the fact that assertions as to the specificity of a compound *in vitro* require rigorous and comprehensive testing in cellular and whole-animal systems.

To the Bedside

The Table is a listing of most of the protein kinase inhibitors currently in clinical trials and the diseases targeted. As can be seen, most are cancer trials, but there is a trend toward targeting protein kinases for the treatment of a number of chronic conditions other than cancer, including inflammatory and cardiovascular diseases. Indeed, several of the agents listed in the Table have strong preclinical data suggesting that they may be efficacious in the therapy of patients with a variety of cardiovascular diseases. The list of potential protein kinase targets for cardiovascular therapies is extensive. However, rather than a summary of disease states and protein kinases possibly involved (an excellent review taking this approach for heart failure was recently published¹⁷), we will discuss a few disease states for which inhibitors exist that are either already in the earliest stages of clinical trials or are in the late stages of preclinical development. These examples, we hope, will illustrate that what was once perceived to be impractical now seems reasonable and attainable.

Acute Coronary Syndromes

Two families of stress-activated MAPKs, the JNKs and p38-MAPKs, are activated by ischemia/reperfusion (I/R).^{14,18} and there is some indication that inhibition of either the JNKs or p38s might prove beneficial for treating acute coronary syndromes (ACS). However, validating these MAPKs as targets in ACS, that is, whether activation of the kinases is beneficial or detrimental, has been difficult.¹⁹ This is because of the lack of good genetic models (ie, mice deleted for the gene) and, until recently for p38, good inhibitors with which to address the question *in vivo*. Two members of the p38 MAPK family, p38 α and p38 β , are activated by ischemia. The first effective inhibitor of p38 α/β was discovered by Lee and coworkers²⁰ at SKF in a broad-based screen for "cytokine-suppressive antiinflammatory drugs" based on their ability to inhibit endotoxin-induced cytokine production by macrophages in culture. The target of this drug was later identified to be the p38s. Because it seems clear that the first wave of drugs targeting kinase pathways to be used in patients will be dominated by p38-MAPK inhibitors, we will

describe these kinases and the mechanisms by which the inhibitors work in some detail.

Preventing the release of inflammatory cytokines and chemokines represents a potentially promising approach to treating ACS (Figure 3) and, possibly, the development and progression of atherosclerotic plaques. Indeed, a p38 inhibitor, VX702, is currently in a phase II clinical trial in patients presenting with ACS. The half-life of the mRNA for many cytokines (and growth factors) is extremely short, allowing for rapid downregulation of expression when the inciting stimulus is removed. This short half-life is largely a result of the presence of AU-rich elements (AREs, consisting of several copies of the sequence AUUUA) in the 3'-untranslated region of the mRNA.²¹ ARE-binding proteins (ARE-BPs) bind to the AREs, and most ARE-BPs target mRNA for degradation. When activated, p38s phosphorylate ARE-BPs, inhibiting their activity.²¹ The end result is p38-dependent stabilization of the cytokine mRNA, leading to increased production of the cytokine protein and activation of inflammatory cells and of endothelial cells, the latter leading to upregulation of adhesion molecules. Thus, p38 inhibitors block phosphorylation of the ARE-BPs, leading to degradation of the cytokine mRNAs, including those coding for TNF- α , interleukin (IL)-1 α/β , IL-6, IL-10, interferon (IFN)- γ , MIP1 α/β , and IL-8. Although stabilization of cytokine mRNA has obviously been an important response to infection over millions of years of evolution, inappropriate activation of inflammatory responses has, over the past 100 years, become a significant factor driving the explosion in the prevalence of a number of chronic disease states.

Several companies have developed p38 inhibitors, and some of these have demonstrated efficacy in models of inflammatory diseases, including inflammatory arthritides and inflammatory bowel disease, as well as in endotoxemia.^{22,23} Some of these inhibitors are currently in clinical trials for rheumatoid arthritis and Crohn's disease (Table). With the rationales that (1) ACSs, including myocardial infarction, had prominent inflammatory components and (2) p38 activation in ischemic tissue might, independent of effects on inflammatory responses, have detrimental effects on cardiomyocyte survival (see Reference 17 and references therein), the efficacy of these drugs was tested in animal models of acute myocardial infarction.

Early-generation p38 inhibitors, SB203580 and SB242710, reduced I/R-induced apoptosis and preserved cardiac function in a Langendorff-perfused rabbit heart model (reviewed in Reference 20). Because with this model, the heart is perfused with a buffer and therefore there are no leukocytes in the perfusate, the findings suggest that p38 inhibition has beneficial effects directly on the myocardium, in addition to its known effects on leukocyte recruitment and activation (Figure 3). This leukocyte-independent protective effect of p38 inhibition on the myocardium probably involves inhibition of I/R-induced production of cytotoxic cytokines by the heart and inhibition of p38-dependent proapoptotic pathways in cardiomyocytes. More recently, a newer-generation p38 inhibitor, SB239063²⁴ (Figure 1), that can readily be used *in vivo* has demonstrated beneficial effects in the intact rat model of I/R injury. In addition to direct protective effects of

p38 on cardiomyocyte survival, SB239063 produced a dramatic reduction in the myocardial inflammatory response, as evidenced by reduced upregulation of P-selectin and intercellular adhesion molecule and reduced neutrophil accumulation within the ischemic zone. Other related potential applications of p38 inhibitors include preservation of mechanical function of cold-stored hearts before transplantation.²⁵ This effect of p38 inhibition may be, in part, related to increased contractility caused by enhanced myofilament responsiveness to calcium.²⁶

There are other potential applications for these cytokine-suppressive drugs, including the treatment of patients with heart failure. Although the RENEWAL and ATTACH trials,²⁷ targeting TNF- α by "capturing" it with a monoclonal antibody or a soluble receptor, produced negative results and raised concerns over worsening of heart failure, this of course does not necessarily mean that the concept of anticytokine therapies in heart failure is invalid, and it is conceivable that more broad-based anticytokine therapy, such as one achieves with p38 inhibitors, could be beneficial. Furthermore, we could benefit from the experiences of the oncologists that demonstrate that one may need to define the molecular phenotype or kinase activity profile of the individual patient, because just as with cancer, patients with the clinical diagnosis of "heart failure" are bound to have very different profiles (as evidenced by the lack of consensus on the signaling abnormalities present in the failing heart¹⁷). Although it is difficult, failing to do so may lead to discarding agents that are effective in subsets of patients. As an example, trastuzumab, the anti-HER2 tyrosine kinase receptor antibody, which confers a 22.5% improvement in overall survival in breast cancer patients with tumors that overexpress HER2 (25% to 30% of all breast cancers), would have been found to be of no value if it had been initially tested in breast cancer patients irrespective of the HER2 status.⁶

Other potential concerns with anticytokine therapies include a possible increased risk of infection, including reactivation of tuberculosis, and the development of opportunistic infections that have been seen with the anti-TNF therapies and with anakinra, an IL-1 receptor antagonist.²⁸ Of course, whether these issues are specific to the anti-TNF and anti-IL-1 therapies used or will be a general feature of all anticytokine therapies remains to be determined.

Stroke

Inhibition of several protein kinase pathways has been shown to be beneficial in animal models of stroke. These include the 3 families of MAPKs, the ERKs, JNKs, and p38 MAPKs.¹⁴ In addition, cell culture studies suggest that inhibitors of glycogen synthase kinase-3 (GSK-3) may also be protective.^{29,30} The first reports of neuroprotection in vivo with a kinase inhibitor used direct injection into the cerebral ventricles of PD98059 (Figures 1 and 2), a first-generation inhibitor of the activation of MEK1/2⁹ (Figure 2 legend), the kinases that activate the ERKs.³¹ This was followed by studies with intravenous administration of another MEK1/2 inhibitor, U0126 (currently in clinical trials for cancer; Table), which was also protective against forebrain and focal cerebral ischemia.³² Remarkably, beneficial effects were seen with

administration after 3 hours of ischemia, before reperfusion. These studies seemed counterintuitive, because the ERKs had generally been thought to be antiapoptotic in most settings (Figure 2), including in I/R injury in the heart.¹⁹ The mechanism of protection may be prevention of excitotoxicity,³³ which is neuronal death caused by release of excitatory amino acids that activate metabotropic glutamate receptors. Excitotoxicity plays a critical role in I/R injury in the brain, and although the precise mechanisms of protection remain to be determined, MEK1/2 inhibitors may be blocking release of glutamate. In addition to stroke, MEK1/2 inhibitors have been reported to be protective against traumatic brain injury.³⁴ As one caveat, PD98059 and U0126 also block activation of MEK5,⁹ the kinase that activates ERK5, the sole member of the fourth MAPK family. Thus, one cannot at this time formally rule out MEK5/ERK5 as the relevant target. Strikingly, another MEK1/2 inhibitor, PD184352, has been reasonably well tolerated when administered orally, twice daily, for 21 days (repeating every 4 weeks) in a phase I dose-ranging trial in cancer patients, with only fatigue, rash, and diarrhea being commonly reported.⁶

Inactivation of JNK3 (via gene deletion in a knockout mouse), which is selectively expressed in the central nervous system, and inhibition of p38 activation (by SB239063) were also protective in stroke models.^{35,36} In the latter case, SB239063 reduced stroke-induced expression of TNF- α and IL-1 β , cytokines that are believed to enhance neuronal loss after I/R. No fewer than 8 companies have reported the development of JNK inhibitors, many focusing on JNK3 and neuroprotection (stroke and neurodegenerative disorders).³⁷ Some have reported enhanced cell survival in a stroke model.³⁷ Safety studies with one agent (CC401, Table) are ongoing in healthy volunteers.³⁷

Inhibitors of GSK-3 are being proposed as potential therapies for disorders as diverse as bipolar mood disorders (lithium and valproic acid are GSK-3 inhibitors), Alzheimer's disease (in which GSK-3 is believed to play a key role in formation of the neurofibrillary tangles and amyloid plaques, the latter being reduced by lithium in an animal model of Alzheimer's disease³⁸), and stroke.³⁰ GSK-3 is inhibited when phosphorylated by the antiapoptotic kinase Akt, and at least part of the antiapoptotic effects of Akt are believed to be mediated by inhibition of GSK-3. GSK-3 inhibition may also mediate part of the phenomenon of ischemic preconditioning.³⁹ Published data are limited, but at this point, selective inhibitors (SB216763 and SB415286; Figure 1) have been shown to block neuronal cell death in culture induced by pharmacological inhibition of the PI3-kinase/Akt pathway or by polyglutamine toxicity caused by the Huntington's disease mutation.^{29,40} Although promising, this kinase is a critical regulator of many basic cellular processes, including development, cardiac growth and hypertrophy, and tumorigenesis.^{41,42} Therefore, it is likely that in the near future, inhibitors of GSK-3 will be restricted to relatively short-term use in high-risk patients.

Hypertension

Rho belongs to a family of small GTP-binding proteins that mediate intracellular signaling induced by activation of het-

erotrimeric G protein-coupled receptors and growth factor receptors. In the cardiovascular system, Rho regulates vascular smooth muscle contraction by modulating sensitivity to Ca^{2+} . One Rho effector is Rho kinase (ROCK), of which 2 isoforms have been identified. ROCKs phosphorylate the myosin-binding subunit of myosin light chain phosphatase and LIM kinase, ultimately regulating phosphorylation of myosin light chain and, via this mechanism, vascular smooth muscle cell contraction.⁴³ Therefore, it is tempting to speculate that ROCK inhibition could enhance coronary vasodilation by changing Ca^{2+} sensitivity of coronary artery smooth muscle cells. In fact, a ROCK inhibitor, hydroxyfasudil, suppresses myosin light chain phosphorylation and significantly inhibits coronary spasm in a pig model. Two recent clinical trials of fasudil indicate that it may be an effective and well-tolerated antianginal agent⁴⁴ and also may be of benefit in patients with microvascular spasm of the coronary arteries.⁴⁵ Although the selectivity of fasudil against ROCKs is in question, these results suggest a potential use of ROCK inhibitors as novel agents to treat symptomatic patients with CAD. Another relatively specific ROCK inhibitor, Y-27632 (Figure 1),⁹ is effective in lowering systolic blood pressure in spontaneously hypertensive rats, DOCA-salt rats, and renal hypertensive rats without affecting blood pressure in normal rats.⁴³ Collectively, selective ROCK inhibitors will probably be a novel approach to the treatment of hypertension. However, Y-27632 has also been shown to affect metastasis, neurite outgrowth, and contraction of smooth muscle cells other than vascular smooth muscle cells.⁴³ Therefore, the safety of Y-27632 and related agents remains a question and will need to be carefully evaluated in clinical trials.

Given the difficulty in controlling hypertension in elderly patients and diabetics, there will probably be many more targets against which inhibitors will be made. These could include the WNK (with no lysine) family of kinases, mutations of which are responsible for a rare hereditary form of hypertension, pseudohypoaldosteronism type II.⁴⁶ Because the WNK4 gene lies close to a locus showing the strongest linkage to blood pressure variation in the Framingham Heart Study, less severe mutations and polymorphisms of the WNK genes may play a more general role in hypertension. If so, these kinases might be ideal targets.

Diabetes and the Metabolic Syndrome

Another avenue open to manipulating activity of protein kinases is to identify drugs that activate, as opposed to inhibiting, a kinase. Protein kinases that are beneficially activated by allosteric mechanisms represent attractive targets for such therapies. One of these is the 5'-AMP-activated protein kinase (AMPK). AMPK exists in the cell as a heterotrimer of α , β , and γ subunits (the α subunit containing the kinase domain). Genetic mutations in the human γ 2 subunit of AMPK have been linked to hypertrophic cardiomyopathy and to ventricular preexcitation.⁴⁷ Specifically, these mutations are associated with a metabolic storage disorder marked by the accumulation of excess glycogen granules in the myocardium. Although the mechanisms by which these mutations lead to cardiomyopathy and preexcitation are not entirely clear, the mutations appear to inhibit

activation of AMPK by AMP. Because AMPK inhibits glycogen synthase, the mutation could lead to increased glycogen synthase activity, increased glycogen production, and the observed accumulation of glycogen in the heart.

The reason that AMPK has generated a tremendous amount of interest on the part of pharmaceutical companies, however, is that activators of it could be useful in the treatment of patients with metabolic syndrome, diabetes, or hyperlipidemia.⁴⁸ AMPK was initially discovered in the early 1970s as an AMP-dependent kinase that inactivated HMG-CoA reductase and acetyl-CoA carboxylase (ACC).⁴⁹ It has since been established that AMPK functions as a cellular "fuel sensor" that is activated in times of reduced energy availability (when [AMP] is relatively high) and serves to inhibit anabolic processes (lipogenesis) and enhance glucose uptake.⁴⁹

Several compelling lines of evidence point to the potential of AMPK as a useful drug target. ACC, the rate-limiting enzyme in fatty acid synthesis, catalyzes the formation of malonyl-CoA, a potent inhibitor of fatty acid oxidation. By inhibiting ACC, AMPK elevates fat oxidation.⁴⁹ In addition, AMPK activation leads to reduced levels of hepatic sterol response element-binding protein-1 and consequently suppresses the expression of several lipogenic genes. Thus, therapeutic activators of AMPK could reduce serum triglycerides. As an inhibitor of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, AMPK also functions to block cholesterol production,⁴⁹ and therapeutic AMPK activators could serve in a manner similar to the statins. In addition, AMPK is activated in exercise, triggering skeletal muscle glucose uptake in an insulin-independent manner. Of particular note, pharmacological activation of AMPK with 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR) mimics exercise and triggers insulin-independent skeletal muscle glucose uptake. Thus, AMPK activators could also alleviate glucose intolerance. In support of this, the biguanide antidiabetic metformin may exert its effects in part by activating AMPK.⁴⁸

The ability to activate AMPK in vitro with AMP and in vivo with AICAR (which is phosphorylated in the cell to ZMP, an analogue of AMP) and the observed antilipogenic and glucose transport effects of AICAR indicate that drugs targeting AMPK will need to be AMPK activators. It is likely that AMP-like compounds will provide the richest source of potential AMPK pharmaceuticals. Identification of such compounds will be assisted by the elucidation of the structural features of the AMPK AMP-binding pocket.

Conclusions

It is very likely that the next several years of translational cardiovascular research will feature a number of clinical trials using inhibitors of protein kinase signaling pathways to treat a variety of disorders. We have touched on some of the targets for which development of inhibitors is more advanced, but there are many others with great potential, including the β -adrenergic receptor kinase (heart failure)⁵⁰ and some kinase inhibitors that are currently in clinical trials for cancer and are in the discovery phase for atherosclerosis and restenosis (eg, growth factor receptors, including the platelet-derived growth

factor receptor, cell cycle regulators such as Cdk-1/-2, and protein kinase C) and for stroke (eg, Cdk).⁵¹ Toxicity remains a major concern, because many of these kinases not only play roles in the pathogenesis of diseases but also function in pathways that regulate the most basic of normal cellular processes. That said, preclinical data have been reassuring. Toxicity data from clinical trials of these agents in cancer will be illustrative, but many of these studies have been designed to identify, or have used, the "maximum tolerated dose," which may be significantly higher than the doses that will be used in cardiovascular diseases. The use of combination therapy, targeting 2 or more kinases on the same or parallel pathways, may allow the use of lower (and therefore less toxic) doses and has shown some promise in cancer trials.⁶ However, the majority of early trials will focus on individual kinases and their role in diseases for which only short-term therapy will be needed (eg, ACS or stroke) or for which targeted local delivery is possible. It must be realized, however, that these may not necessarily be the disease states most likely to benefit from therapy. Finally, as highlighted above, given the vast numbers of protein kinases in the human genome and their sequence and structural similarities, added to the inability to test the drugs against all kinases, specificity will remain a concern with these agents. Despite these obstacles, this new class of agents offers a great deal of promise to expand our therapeutic options for a wide variety of cardiovascular diseases.

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